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Biological Effects of Long-Term Exposure of Rats to 970-MHz Radiofrequency Radiation

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Rats (N = 16) exposed individually in circularly polarized waveguides to 970-MHz electromagnetic radiation (SAR = 2.5 mW/g, 22 h daily for 70 consecutive days) had significantly higher serum levels of triglycerides, albumin, and total protein compared with sham-irradiated controls. No difference was observed in the weights, hematologic profile, or in vitro lymphocyte responses to mitogens between these two groups. The higher serum levels of triglycerides in radiofrequency-radiation-exposed rats suggest a nonspecific stress reaction.

Key words: 970 MHz, rats, long-term exposure, serum chemistry, hematology, lymphocytes.

The biological effects of acute exposure to radiofrequency (RF) radiation have been studied in many laboratories. On the other hand, few experiments of long-term exposure to RF radiation have been conducted. The purpose of this study was to determine effects on the blood and blood cells of rats exposed to 970 MHz for 22 hours daily for 70 consecutive days.

The RF exposure system [Weil, 1980] consists of 16 individual circularly polarized (CP) waveguides that operate at 970 MHz. Sixteen similar nonenergized chambers served for sham-irradiation controls. The waveguide design is based on that described by Guy and Chou, [1975] but incorporates the following modifications: 1) a smaller waveguide diameter, ie, 1.3 cm less than described by Guy and Chou [1975]; 2) slug tuners for each waveguide; and 3) hybrid 3-db couplers (Microlab/ FXR CA-35N) rather than hybrid rings for CP production. The waveguides are fed from a single (AIL 125 Cutler-Hammer, Long Island, NY) RF

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power generator (60 W output) via a 4 × 4 power dividing network consisting of a single four-way divider (Microlab D4-17N) and four custom-made four-way dividers having a minimum of 20 db isolation between output ports (Struthers Electronics Corp, Farmingdale, NY). Adequate isolation in the latter dividers is essential to reduce variations of input power to each waveguide.

The power absorbed in one of the loaded waveguides was continuously monitored over the 22-h exposure period using the same technique described by Guy and Chou [1975]. This information was fed to a microcomputer system that compiled data on the mean incident power and power absorption for the unit, as well as average whole-body specific absorption rate (SAR). All 16 of the energized waveguides were sequentially monitored, one waveguide per day, several times during the experiment. The nominal input power per waveguide was 2.5 W giving an "actual" incident power density, averaged across the waveguide, of approximately 7.8 mW/cm^2 (equivalent to an "effective" or far-field power density of 25 mW/cm^2 as described by Guy and Chou [1975]. The average whole body SAR during the course of the experiment was 2.5 ± 0.3 mW/g, (X ± SD, N = 43).

Rats (14-week-old, male Wistar-Furth, Microbiological Associates, Bethesda, Md) were held in vented circular acrylic cages that fit into the waveguides. Food and water were available ad libitum using a system identical to that described by Guy and Chou [1975]. The rats were maintained on a 12 h light-dark cycle. Rats were randomly assigned to the CP and sham waveguides four weeks prior to the start of the RF irradiation so that they would acclimate to the system. All of the rats were in a single room of the vivarium in which the CP and sham waveguides were mounted alternately on cage racks. During the experiment, the room temperature and relative humidity were maintained at 21–24 °C and 50%–75%, respectively. Power to the waveguides was switched off for two hours daily so that food and water could be replenished. On Mondays, Wednesdays, and Fridays, all cages were cleaned, and all rats were weighed. No significant difference (analysis of variance) was observed in the mean weight of irradiated compared with sham-irradiated rats during the portion of the acclimatization period immediately prior to the start of irradiations, or during the irradiations with 970 MHz (see Fig. 1).

The rats were irradiated for 69–70 consecutive days for a total of over 1,500 hours. Eight of the RF- and eight of the sham-irradiated rats were bled from the aorta while under ether anesthesia on day 69 of irradiation while the remaining animals were bled on day 70. The animals received no food for 18 hours prior to bleeding. Approximately 10 ml of blood was collected from each rat, immediately after which the animals were killed by cervical dislocation and their spleens aseptically removed. The total time for anesthetization, bleeding, and spleen removal was less than five minutes per rat. Blood smears were prepared on slides with Wright's stain and 200 leukocytes were differentially counted for lymphocytes, monocytes, eosinophils, and polymorphonuclear leukocytes. Erythrocyte count, leukocyte count, mean cell volume of erythrocytes, hematocrit, and hemoglobin concentration of heparinized blood (1 ml of total volume) were evaluated on a Coulter counter (Model ZBI-6, Coulter Electronics, Inc, Hialeah, Fla). No significant difference (Student's t-test) was observed between sham and irradiated rats for any of these hematologic indices.

The remaining blood was placed in sterile tubes and allowed to clot. Serum was removed and immediately frozen at −20 °C. The sera were shipped frozen in dry ice to Smith-Kline Clinical Laboratories, Inc, Creve Coeur, Mo, for standard analysis with their SMAC® panel. The levels of the following serum components were determined for each rat: glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, chloride, uric acid, calcium, phosphorus, cholesterol, triglyceride, alkaline phosphatase, aspartate transaminase (SGOT), alanine transaminase (SGPT), creatinine phosphokinase (CPK), lactic dehydrogenase (LDH), albumin, globulin, and total protein. Significant ($P < 0.05$, analysis of variance) elevations in serum triglyceride, albumin, and total protein concentrations were observed in irradiated compared with sham-irradiated rats (Table 1). Analysis of variance indicated a significant correlation between treatment and elevation in these serum components, as well as a correlation of these increases within individual animals [Barr et al, 1976]. No other serum components were significantly different between these two groups.

Triplicate microcultures of spleen cells to which were added different mitogens (phytohemagglutinin, concanavalin A, pokeweed mitogen, or Escherichia coli lipopolysaccharide) were prepared and the amount of ^{3}H-thymidine incorporated into DNA was determined after 72 hours of culture [Smialowicz et al, 1979]. Phytohemagglutinin and concanavalin A stimulate T (thymus-derived) lymphocytes, lipopolysaccharide stimulates B (bursa-equivalent) lymphocytes, whereas pokeweed mitogen stimulates both T and B lymphocytes. No significant difference was observed in the in vitro mitogen-stimulated response of splenic lymphocytes from sham and 970-MHz irradiated rats for any of these mitogens.

The increases in albumin and total protein in irradiated rats are difficult to explain in the absence of changes in erythrocyte assays, such as hematocrit, hemo-

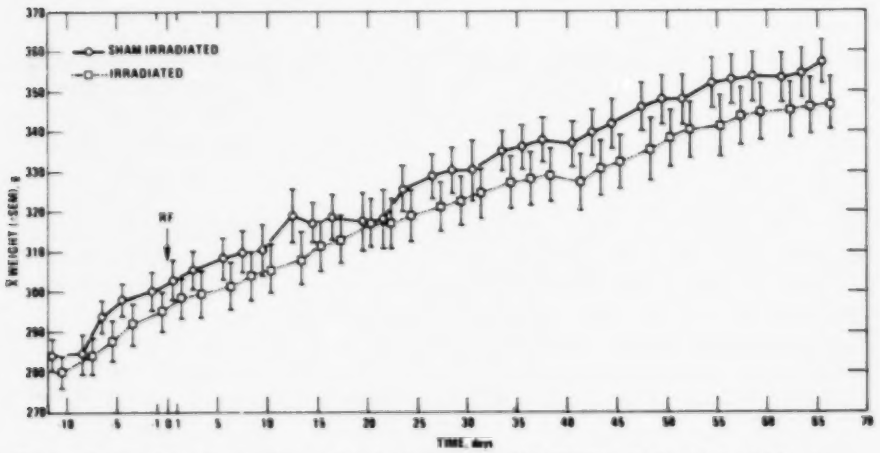

Fig. 1. Body weight of rats before and during irradiation with 970 MHz. RF irradiation initiated on day 0 (1).

TABLE 1. Serum Chemistry Data for Rats Irradiated with 970 MHz, 22 h Daily for 70 Consecutive Days

Test (units)	Sham-irradiated (N = 16) (Mean ± SD)	Irradiated (N = 16) (Mean ± SD)
Glucose (mg/dl)	127 ± 3	122 ± 2
BUN (mg/dl)	19.6 ± 1.4	18.8 ± 1.1
Creatinine (mg/dl)	0.5 ± 0.01	0.6 ± 0.01
BUN/Creatinine (ratio)	37 ± 2.3	33 ± 1.9
Sodium (mEq/L)	144 ± 0.3	145 ± 0.5
Potassium (mEq/L)	4.7 ± 0.1	4.6 ± 0.2
Chloride (mEq/L)	107 ± 0.3	107 ± 0.5
Uric acid	1.6 ± 0.1	1.7 ± 0.2
Calcium (mg/dl)	9.4 ± 0.1	9.5 ± 0.1
Phosphorus (mg/dl)	4.8 ± 0.1	4.7 ± 0.1
Cholesterol (mg/dl)	67 ± 2	63 ± 1
Triglyceride (mg/dl)	56 ± 3	75 ± 3[a]
Alkaline phosphatase (mU/ml)	129 ± 9	127 ± 7
SGOT (mU/ml)[c]	137 ± 3	137 ± 3
SGPT (mU/ml)	35 ± 3	30 ± 3
CPK (mU/ml)	906 ± 31	983 ± 50
LDH (mU/ml)[c]	3125 ± 280	2565 ± 150
Total protein (g/dl)	6.06 ± 0.03	6.16 ± 0.02[a]
Albumin (g/dl)	3.69 ± 0.02	3.76 ± 0.02[b]
Globulin (g/dl)	2.37 ± 0.02	2.40 ± 0.01
A/G (ratio)	1.56 ± 0.02	1.57 ± 0.02

[a]$P < 0.01$, analysis of variance.
[b]$P < 0.05$, analysis of variance.
[c]Values abnormally high, reference values for SGOT and LDH are 58–94 and 41–102 mU/ml, respectively.

globin, or red cell count, which would indicate that these animals may have been dehydrated. The values reported here for albumin and total protein are within the normal range for Wistar rats [Mitruka and Rawnsley, 1977]. The fact that elevations in these serum components were found to be correlated with increases in triglycerides, by statistical analysis [Barr et al, 1976], suggests some causal relationship.

The observed increase in serum triglycerides is also difficult to explain in the absence of changes in weight, or in other serum components such as glucose and cholesterol, which might implicate a change in diet (ie, food intake). On the other hand, it is known that stress causes an increase in triglyceride levels, as does the administration of glucocorticoids [Bagdade et al, 1976; Reaven et al, 1974]. Furthermore, it has recently been reported that 2,450-MHz microwave radiation caused an increase in serum triglyceride levels in mice [Deficis et al, 1979]. Increases in serum triglyceride levels were correlated with increases in β-lipoprotein levels in serum of microwave-irradiated mice, however, no change was observed in cholesterol levels [Laurens et al, 1979]. These investigators concluded that the observed increase in triglyceride and β-lipoprotein levels in mice exposed to 2,450 MHz was due to stress produced by microwave heating. Whether the in-

crease in triglyceride and protein levels in the present study is due to an RF-induced stress response remains to be determined.

The rats exposed to 970 MHz in this study absorbed RF energy at an average dose rate of approximately 2.5 mW/g. This rate of energy absorption is approximately equal to one half of the basal metabolic rate of an adult rat [Kleiber, 1975]. In endotoxin-induced hypothermic rats, this rate of energy absorption is sufficient to cause a 2 °C increase in colonic temperature after a 90-minute exposure to 2,450 MHz [Smialowicz et al, 1980]. Based on these considerations, it appears that the rats irradiated with 970 MHz received a thermal load from the RF energy. Whether this heating was responsible for producing the observed effects requires further investigation, to both validate the observed results, and to determine the mechanism by which these changes occur. A replicate of this study is planned to determine if these results can be corroborated, and to evaluate other indicators of a stress-induced response.

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DISCLAIMER

This report has been reviewed by the Office of Research and Development, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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